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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : **G01N 24/08, 24/12, 33/53**	**A1**	(11) International Publication Number: **WO 98/57155** (43) International Publication Date: 17 December 1998 (17.12.98)

(21) International Application Number: PCT/US98/12393

(22) International Filing Date: 15 June 1998 (15.06.98)

(30) Priority Data:
60/050,060   13 June 1997 (13.06.97)   US
09/022,022   11 February 1998 (11.02.98)   US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS FOR IDENTIFYING DRUG CORES

(57) Abstract

The present invention relates to methods for detecting chemical moieties that may serve as the core or scaffold of a potential drug that is directed to a target.

METHODS FOR IDENTIFYING DRUG CORES

TECHNICAL FIELD OF THE INVENTION

The present invention relates to methods for detecting chemical moieties that may serve as the core or scaffold of a potential drug that is directed to a target. The invention further relates to a chemical library of drug cores and the use of that library to identify useful drug cores for a particular target protein.

BACKGROUND OF THE INVENTION

In a target directed drug discovery program, there are many different strategies that may used to identify a clinical candidate. Although these different approaches to drug discovery might follow significantly different pathways of optimization to a highly potent and bioavailable drug molecule, they all share a common origin: they must begin with a lead compound. Many of the properties of the final compound or class of compounds, e.g. target affinity, inhibitory potential, solubility, and bioavailability, may be inextricably tied to those of the initial lead compound. Therefore, the methods by which leads are identified in the early stages

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could significantly impact the success of the project in
the latter stages.

Lead molecules are currently identified and
selected in a number of different ways. A lead molecule
5   may be a known drug molecule, or an analog of a known
drug. Alternatively, if the target is an enzyme, the
lead may be a substrate or substrate analog. Often, a
lead is discovered by random screening of either
commercially available or proprietary compound libraries,
10   or both. However, there are many potential problems
which may arise using the above strategies. For example,
starting with a competitor's drug may not lead to
sufficient diversity in the final class of compounds to
avoid impinging upon that competitor's intellectual
15   property.

Starting with a bioactive natural product may
necessarily force the design of large molecular weight
analogs, with poor synthetic accessibility and difficult
scale-up problems. Alternatively, using random screening
20   to generate leads might result in a novel, synthetically
accessible class of compounds, but unless several
multiple hits from random screening are optimized in
parallel, the compound class might lack sufficient
diversity to overcome problems related to solubility or
25   bioavailability without overly compromising potency.

When random screening is used to generate leads
in a drug design program, there are several approaches
which may be taken. A brute force approach is to screen
very large (>100,000) numbers of compounds, identify a
30   potent binder or inhibitor of the drug target, and then
modify that binder to optimize its activity against the
drug.

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In a structure-based program, a more rational approach is to start by using information-driven methods for virtual screening of databases to select a smaller subset of compounds for high throughput screening. This
5   approach requires that structural information about the target and particularly its binding site(s) be available.

Computer modeling is then routinely employed to search available databases for good screening candidates. Then, a subset of representative compounds is assayed for
10   binding/inhibition. Weak binders/inhibitors then become leads for interative structure based drug design or SAR, depending on what structural information regarding the target is available.

The downside of this approach is that in
15   choosing representative compounds for actual binding/inhibition studies one may miss potentially important leads. This may occur because too small a number of representative compounds were chosen for screening or that there was insufficient diversity in
20   that representative set.

Thus, there is still a need for improved methods of identifying lead compounds. Moreover, it would be particularly advantageous if the generation of lead compounds could be efficiently and accurately
25   achieved without the need for obtaining complex structural information about the target.

Another important aspect of lead generation is the ability to detect compounds that bind weakly to the target. Such compounds include those that bind to the
30   active site of an enzyme, but do not inhibit its enzymatic function. Lead compounds such as these go undetected in enzyme inhibition assays. Moreover, many

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of the standard assays used to detect binding have a
limit of detection in the micromolar range. However,
many lead compounds that bind in the millimolar range may
have desirable properties (e.g., ease of synthesis, good

5   solubility, good bioavailability) and can ultimately be
optimized to significantly increase binding.

         The use of one type of NMR technology to design
and identify weakly binding compounds is disclosed in PCT
Publication Nos. WO 97/18471 and WO 97/18469. The NMR

10   technology disclosed in these documents, however, suffers
from several shortcomings. First, it can be only be
applied to targets of low molecular weight (< 20 kDa).
Second, it requires isotopic labeling of the target which
is both expensive and usually results in lower yields of

15   proteins. Third, and most important, that technique
requires that the x-ray crystal or NMR structure of the
target be solved prior to employing the method.

         Thus, there is still a need for techniques
which can detect weak binding of ligands to targets which

20   are either large in size and/or for which no structural
information is available.

                   SUMMARY OF THE INVENTION

         The present invention solves the problems
25   indicated above by providing a method for detecting weak
binding of potential drug cores to targets, regardless of
the size of the target and without requiring x-ray
crystallographic or NMR structural information about the
target.

30   The method of this invention preferably
incorporates the use of NMR to detect binding,
particularly the techniques known as transferred nuclear

Overhauser effect ("tNOE"), differential line broadening
("DLB"), relaxation filtering, and pulsed-field-gradient
NMR spectroscopy ("PFG NMR").

5   In this method, a single compound selected from
a chemical library of known drug cores or a mixture of
such compounds is combined with a target and subjected to
NMR. The ligand or ligands in this mixture which bind
weakly to the target bind to and come off the target
numerous times during the NMR procedure. NOEs built up
10   by these ligands in the bound state are transferred to
the ^{1}H NMR signals of excess free ligand. As a result,
the relative signs of diagonal and cross peaks in the
spectrum changes with respect to those observed for the
free ligand alone, thus providing an unambiguous
15   indication of binding.

       Similarly this rapid equilibrium between the
bound and the unbound state creates a characteristic
decrease in amplitude of peak heights and, in most cases,
a broadening of one or more peaks in the one-dimensional
20   NMR spectrum of the ligand. This, too, can provide an
unambiguous indication of binding in the methods of this
invention.

       In PFG NMR, the diffusion coefficient of the
drug core alone and in the presence of the target are
25   compared. If the core binds to the target, than the
detected diffusion coefficient of the drug core is
reduced. The amount by which that coefficient is reduced
can be used to calculate a K_d value.

       Once identified as being capable of binding to
30   the target, these drug cores can be modified and
optimized by the addition of side groups to create a
potential drug candidate. In addition, two or more drug
cores that display binding to the target can be combined

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into a single molecule to optimize and increase binding affinity.

The invention also provides a relatively small library of soluble carbocyclic and heterocyclic ring
5   systems which represent frameworks most commonly found in known drug molecules. Some of these frameworks have been described by G. W. Bemis et al., <u>J. Med. Chem.</u>, 39, pp. 2887-2893 (1996). These rings optionally contain one or more of a small group of side chains which are also
10   commonly present in commercially available drugs. The advantage of this library is its small size and its heavy bias towards being "druglike."

The term "druglike", as used herein, means properties that are considered important for commercial
15   drugs. These include solubility, bioavailability, ease and low cost of synthesis (including low cost of starting materials and the ability to produce the final product using few synthetic steps), low toxicity, and chemical and metabolic stability.

20   Because the library of this invention consists of cores and side chains that are present in commercially available drugs, any weak binders detected therein will necessarily have desirable druglike qualities. Moreover, the small size of the library makes screening less time
25   and labor intensive. Finally, the diverse nature of the cores and side chains in the library means that multiple "hits" (i.e., weak binders) are likely, allowing the flexibility and advantages of pursuing several compound classes at once.

30   Once one or more members of the library are identified as weakly binding a given target, each binder is used to bias the clustering of large chemical databases (either commercially available or proprietary)

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and to select a group of compounds for high throughput
screening.

<u>BRIEF DESCRIPTION OF THE DRAWINGS</u>

Figure 1, panel A, depicts the one-dimensional
5   proton NMR spectra of a mixture of nicotinic acid (peaks
indicated by an 'X') and 2-phenoxy benzoic acid (peaks
indicated by a 'Y') in the presence of the target, p38.
Figure 1, panel B, depicts the one-dimensional proton NMR
spectra of a mixture of nicotinic acid and 2-phenoxy
10   benzoic acid in the absence of target. A relaxation
filter was used after the preparatory delay to attenuate
broad resonances arising from the protein.

Figure 2, panel A, depicts the 2D NOESY spectra
of a mixture of nicotinic acid ('X') and 2-phenoxy
15   benzoic acid ('Y') in the absence of target. Figure 2,
panel B, depicts the 2D NOESY spectra of a mixture of
nicotinic acid and 2-phenoxy benzoic acid in the presence
of p38 MAP kinase.

Figure 3, panel A, depicts the ω_2 cross-
20   sections from the NOESY spectra shown in Fig. 2. of the
mixture of nicotinic acid ('X') and 2-phenoxy benzoic
acid ('Y') in the absence of target. Figure 3, panel B,
depicts the ω_2 cross-sections from the NOESY spectra
shown in Fig. 2. of the mixture of nicotinic acid ('X')
25   and 2-phenoxy benzoic acid ('Y') in the presence of p38
MAP kinase.

Figure 4 depicts the Water-sLED pulseq sequence
for measuring translational diffusion coefficients.
Proton 90° rf pulses and solvent flip-back pulses are
30   indicated on the top staff by the black vertical bars and
unfilled domes, respectively. Gradients are given on the

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lower staff and are applied along the Z-axis. Only the
phase-encoding and phase-decoding gradients are shaded.
The gradients are of length δ = 4ms, and their strengths
are identical. The strengths are varied parametrically

5   in a series of one-dimensional experiments. "Δ"
indicates the total time between the two gradients.
During the "T" period (50 ms), phase-encoded
magnetization is aligned with the external magnetic
field.

10   Figure 5 depicts an example of signal decay in
the water-sLED experiment. Gradient strength increases
from left to right.

            Figure 6 depicts fits of the peak integrals
versus $K^2 = \gamma^2\delta^2G_z^2(\Delta-\delta/3)$ to determine the diffusion

15   coefficients. Peak integrals for free 2-phenoxybenzoic
acid, 2-phenoxybenzoic acid in the presence of p38, and
the p38 aromatics, correspond to the open squares, filled
diamonds, and filled circles, respectively.

            DETAILED DESCRIPTION OF THE INVENTION

20   Based upon a survey of molecular shapes which
represent frameworks most commonly found in known drug
molecules, applicants have designed a library of what we
refer to as "drug cores." Without being bound by theory,
we believe that the prevalence of these drug cores in

25   known drug molecules is due, at least in part, to the
fact that they impart desirable properties to a drug
molecule. These properties include solubility,
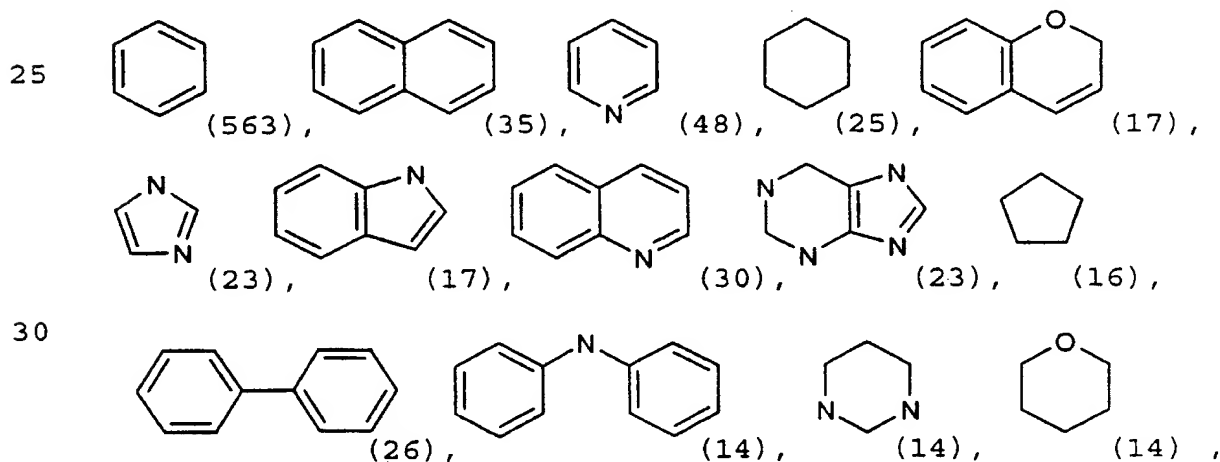bioavailability, lack of toxicity, etc.

            We believe that a drug design effort that

30   begins by identifying a drug core within our small, but
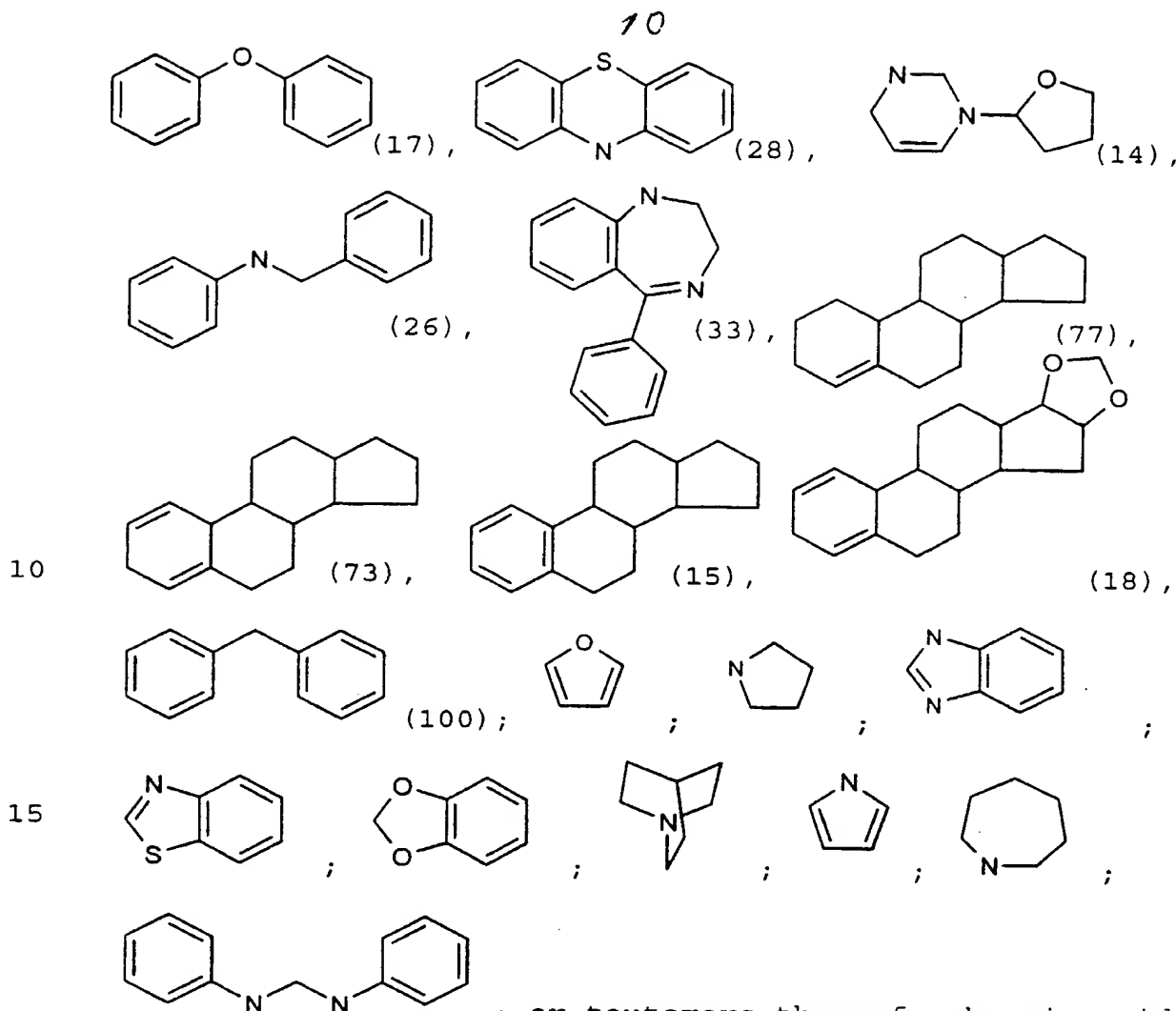diverse library of low molecular weight, soluble drug

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cores that binds to a desired target and then builds off
of that target (e.g., by adding additional substituents)
has a better likelihood in producing a safe and effective
drug than other techniques, such as screening thousands

5   of members of a combinatorial library.

The problem that one faces in identifying a
drug core that binds a target is that, at best, the cores
bind the target with a K_d in the μM to mM range. At that
binding affinity most of these cores would be missed in a

10   standard enzymological inhibition assay. Applicants have
discovered a way around this problem by applying the well
known nuclear magnetic resonance ("NMR") techniques of
differential line broadening ("DLB"), relaxation
filtering, the transferred nuclear Overhauser enhancement

15   ("tNOE") and pulsed-field gradient NMR ("PFG"). These
techniques may be applied with no limitation on the size
of the target and no requirement for isotope labeling of
the target to detect the binding of drug cores.
Thus, according to one embodiment, the invention provides

20   a method of identifying a drug core suitable for a given
target comprising the steps of:

a)   providing a drug core consisting of a
cyclic structure selected from:

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(563), (35), (48), (25), (17),

(23), (17), (30), (23), (16),

30

(26), (14), (14), (14) ,

10

(17),   (28),   (14),

(26),   (33),   (77),

10   (73),   (15),   (18),

(100);   ;   ;   ;

15   ;   ;   ;   ;   ;

; or tautomers thereof, wherein said
20   cyclic structure is optionally substituted at:

      i)   one or more carbon atoms with one or
more substituents independently selected from $=O$,
$-CH_3$, $-OH$, $-OCH_3$, $-Cl$, $-NH_2$, $-C(O)OH$, $-F$, $-CH_2OH$,
$-CH_2CH_3$, $-OC(O)CH_3$, $-NO_2$, $-N(CH_3)_2$, $-CF_3$, $-C(O)NH_2$,
25    $-C(O)OCH_3$, $-C(O)OCH_2CH_3$, $-CH(CH_3)_2$, $-S(O)_2NH_2$,
$-C(O)CH_3$, $-CN$, $-Br$, $-I$, $-S(O)_2OH$, $-OCH_2CH_3$,
$-CH_2C(O)OH$, $-OC(O)CH_2CH_3$, $-CH_2CH(CH_3)_2$, $-C(O)CH_2OH$,
$-N(H)C(O)CH_3$, $-C(CH_3)_3$, $=S$, $-CH_2NH_2$,
$-OCH_2CH(OH)CH_2N(H)C(CH_3)_3$, $-N(H)CH_3$, $-CH(CH_3)C(O)OH$,
30    $-C\equiv CH$, $-(CH_2)_2CH_3$, $-CH_2C(O)NH_2$,
$-OCH_2CH(OH)CH_2N(H)CH(CH_3)_2$, $=N-OCH_3$, or $-OCH_2CH_3$

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ii)   one or more nitrogen atoms, if
present, with a substituent independently selected
from $-CH_3$, $-(CH_2)_2OH$ or $-CH_2CH_3$;

iii) a sulfur atom, if present, with $=O$; and

5   b)   determining whether any one of said drug cores
binds to said target.

The term "target", as used herein, refers to
any biologically important molecule which is capable of
binding to another molecule. The term includes proteins,
10 particularly enzymes, peptides, nucleic acids, such as
DNA and RNA, membrane proteins in detergent or micelles,
subcellular structures or organelles, any of the
foregoing attached or tethered to a solid support, or any
of the foregoing already bound to a ligand.

15   A molecule that is already bound to a ligand
(e.g., one of the drug cores of this invention) is
expected to have sufficient room in its binding pocket to
bind additional ligands, such as a second drug core of
this invention. In this manner, multiple drug cores that
20 display binding to the target molecule can be combined
(and subsequently modified through the addition of
substituents) to create a potential drug.

The solvent into which the protein and target
are mixed can be any solvent in which both the target and
25 drug core are soluble and stable and which is compatible
with NMR or other techniques useful to detect binding.
Most preferably, the solvent is an aqueous buffer system.

The choice of drug core will depend upon what,
if any, structural information one has about the target.
30 If some structural information is available about the
shape or nature of the binding site, one will select
those drug cores that have the requisite shape, size and

/2-

nature to theoretically fit into and interact with that
binding site.

If nothing is known about the target, the
choice of drug core should be made simply by prevalence
5   of that core in known drugs. The numbers in parentheses
below certain of the drug cores depicted above reflects
the number of commercially available drugs listed in the
Comprehensive Medicinal Chemistry ("CMC") database
(version 94.1) that contain that core. These numbers
10   exclude those compounds which are radiopaque agents,
contrast agents, solvents, anesthetics, disinfectants,
topicals, local agents, spermicides, wetting agents,
flavoring agents, pharmaceutical aids, surgical aids,
dental compounds, surfactants, sunscreens, ultraviolet
15   screens, emetics, preservatives, aerosol propellants,
chelators, keratolytics, insecticides, astringents,
herbicides, laxatives, sweeteners, dental caries
prophylactics, adhesives, veterinary compounds, buffers,
scabicides and ectoparasiticides. Thus, one would start
20   with the drug core having the highest number in
parentheses and, in order, work their way down to the
cores that are least prevalent, followed by those cores
that do not contain any parenthetical numbers.

More preferably, the cyclic portion of the drug
25   core is a drug core that is prevalent in known drugs and
is selected from:

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5

15

, or
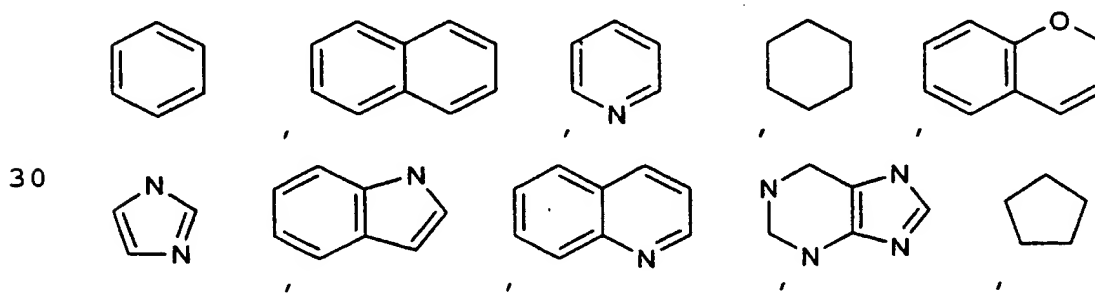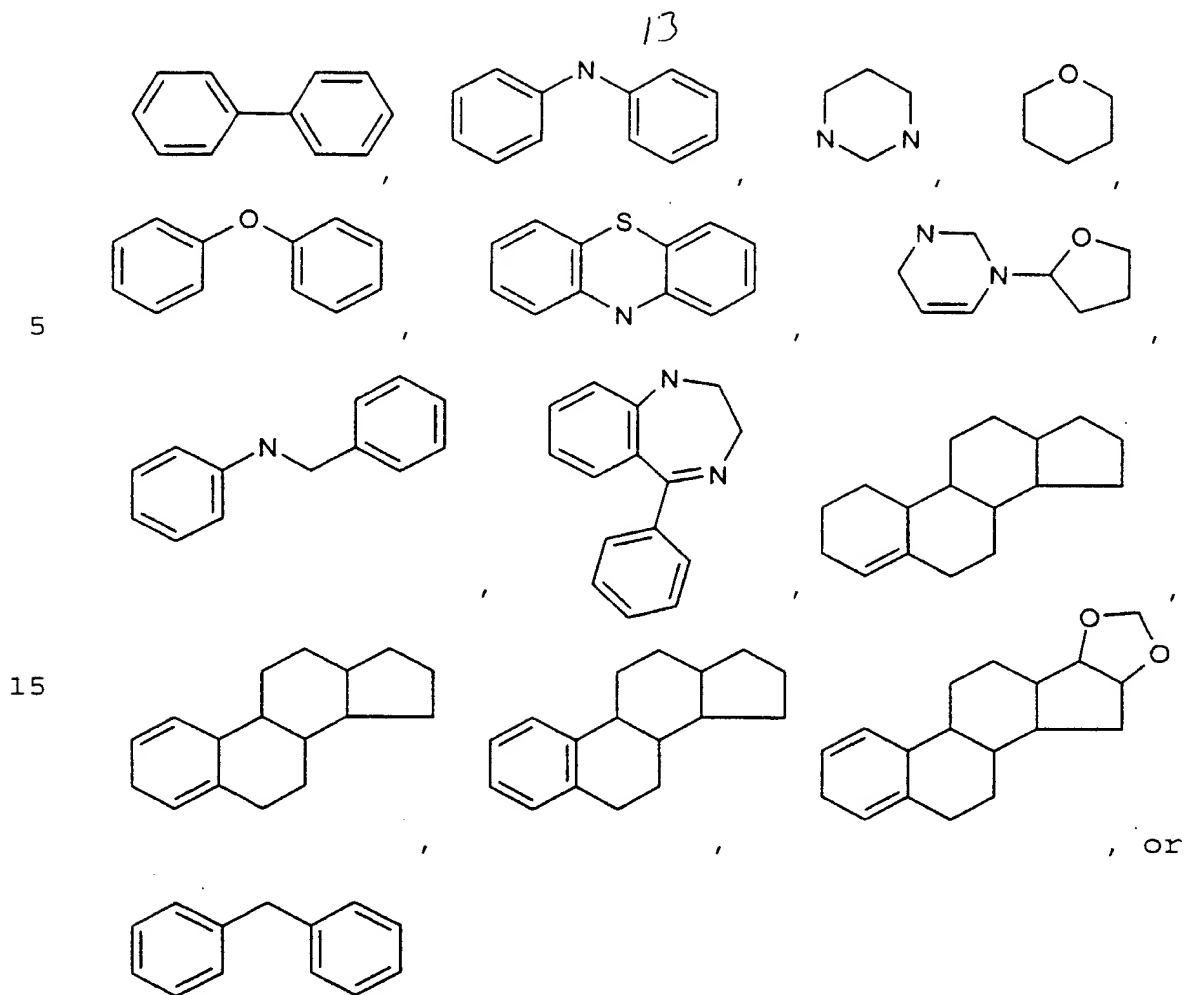
25   The choice of substituents to place on the drug core is dependent upon two factors: prevalence of those side chains in existing drugs, and the solubility of the drug core in the absence of substituents. In other words, the substituent should be chosen so that the

30   resulting drug core is soluble in the solvent system being used. Those of skill in the art will recognize which of the cyclic structures depicted above will be insoluble in a given solvent system and which substituents will impart increased solubility to that

35   cyclic structure.

        More preferably, the optional substituents attached to a carbon atom are independently selected from

-CH$_3$, -OH, -OCH$_3$, -Cl, -NH$_2$, -C(O)OH, -F, -CH$_2$OH, -CH$_2$CH$_3$,
-OC(O)CH$_3$, -NO$_2$, -N(CH$_3$)$_2$, -CF$_3$, -C(O)NH$_2$, -C(O)OCH$_3$,
-C(O)OCH$_2$CH$_3$, -CH(CH$_3$)$_2$, -S(O)$_2$NH$_2$, -C(O)CH$_3$, -CN, -Br, -I,
-S(O)$_2$OH, -OCH$_2$CH$_3$, -CH$_2$C(O)OH, -OC(O)CH$_2$CH$_3$, -CH$_2$CH(CH$_3$)$_2$,

5   -C(O)CH$_2$OH, -N(H)C(O)CH$_3$, -C(CH$_3$)$_3$, =S, -CH$_2$NH$_2$,
-OCH$_2$CH(OH)CH$_2$N(H)C(CH$_3$)$_3$, -N(H)CH$_3$, -CH(CH$_3$)C(O)OH, -C≡CH,
-(CH$_2$)$_2$CH$_3$, -CH$_2$C(O)NH$_2$, -OCH$_2$CH(OH)CH$_2$N(H)CH(CH$_3$)$_2$ or
=N-OCH$_3$.

          Most preferably, the substituents attached to a

10   carbon atom are independently selected from =O, -OCH$_3$,
-OH, -NH$_2$, -C(O)OH, -S(O)$_2$OH, -S(O)$_2$NH$_2$, -CH$_2$OH or
-C(O)NH$_2$; the substituent attached to a nitrogen atom is
CH$_3$; and the substituent attached to a sulfur atom is =O.
These most preferred substituents are both widely

15   prevalent in drugs and impart solubility in aqueous
buffers to the ring system to which they are attached.

          While the invention envisions that the drug
cores can contain any number of substituents that is
chemically feasible, it is preferred that the number of

20   substituents be from 0 to 3.

          The final step of the method involves
determining whether the drug core binds to said target.
It is believed that the practical limit of detection of
binding requires a K$_d$ of less than about 10 millimolar.

25   As a practical matter, the drug cores listed
above are more soluble in organic solvents than in
aqueous solution. Thus, they are stored in an organic
solvent, such as DMSO, prior to mixing with the target.
The target is typically stored in solid form or in an

30   aqueous solution. When the drug core is mixed with the
target, the drug core solvent and the target solvent are
usually miscible. Because the volume of solvent

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containing the drug core is far less than the volume of
aqueous solution containing the target, even when the two
solvents are not miscible, the drug core will transfer
from the organic phase to the aqueous phase, thus
5   allowing both the drug core and the target to reside in
the aqueous phase.

As set forth above, it is preferred that NMR
techniques be used to detect the weak binding of the drug
cores to the target. Other, less preferred, methods of
10   detecting such binding include functional activity
assays, immunoreactivity, radiological assays such as
scintillation proximity and competition with radioactive
tracers, spectral methods such as ultra-violet, visible,
infrared and fluorescence spectroscopy, circular
15   dichroism, surface plasmon resonance, calorimetry, mass
spectrometry, liquid chromatography and equilibrium
dialysis.

According to one preferred embodiment, the
determination of binding is achieved by the NMR method of
20   line broadening, relaxation filtering or a combination of
the two and comprises the steps of:

i)   obtaining a one-dimensional NMR spectrum
of said drug core in the absence of said target;

ii)   mixing the target with the drug core at a
25   molar ratio of between 1:1 and 1:100.

iii) subjecting said mixture to nuclear
magnetic resonance for a period of time sufficient to
obtain a one-dimensional spectrum; and

iv)   comparing the spectra obtained in steps i)
30   and iii) to determine if said drug core has bound to said
target.

If binding between the drug core and target has
occurred, the width of one or more of the drug core peaks

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in the drug core + target spectrum will increase as
compared to the drug core alone spectrum. Similarly, the
amplitude of one or more of the peaks corresponding to a
binding drug core will decrease. Preferably, the two
5   methods are used in conjunction wherein the sample is
first subjected to a relaxation filter when generating
the one-dimensional spectra. This serves to filter out
the resonances of the target in the drug core + target
spectrum and makes interpretation of line broadening
10   easier. Also, with drug cores that bind in the
millimolar range, line broadening may be minimal and not
easily detectable. such weak binding drug cores will,
however, demonstrate a more easily detectable decrease in
peak amplitude caused by the relaxation filter.
15   An advantage of this technique is that multiple
drug cores can be tested in the same sample. In testing
multiple drug cores in the same sample, it is preferred
that at least one peak in each drug core spectrum be
non-overlapping with all of the other peaks from the
20   other drug cores in a one-dimensional spectra. This is
so, because when one observes line broadening, one must
be able to identify which drug core corresponds to the
broadened peak. However, even if all peaks of a
particular drug core are overlapped by peaks of other
25   drug cores in the sample, one can still detect binding
using subtraction methods. These methods involve
subtracting the spectrum obtained from the mixture of
drug cores in the absence of target from the spectrum
obtained in the presence of the target. Peaks
30   corresponding to drug cores that bind to the target will
be visible after the subtraction, while non-binding drug
core peaks will be obliterated.

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Moreover, when one tests multiple drug cores in a single sample, one should obtain a reference spectrum of the combination of drug cores in the absence of the target, as well as reference spectra for each individual

5   drug core.

The other requirements are that 2 or more of the drug cores in said sample do not interact chemically with one another, with the NMR solvent system, or with the NMR buffer components utilized to determine binding.

10   This is important because the structure of the reacting drug cores will be altered and will not reflect the structure that one desires to test for binding. Also, the products of the reaction may have different NMR spectra, therefore making interpretation of line

15   broadening difficult or impossible. Those of skill in the art will know, based upon drug core structure and buffer conditions to be used in NMR whether 2 or more drug cores would be expected to react with one another.

Lack of interaction also means that the drug

20   cores in the sample should not aggregate or induce precipitation with one another or the target, nor bind covalently to one another. These adverse events will increase the perceived molecular weight of one or more drug cores or the target in the sample or remove them

25   from solution.

Thus according to another embodiment, the determination of binding is performed on a sample containing multiple drug cores mixed with a single target and is achieved by the NMR method of line broadening

30   comprising the steps of:

i)   obtaining one-dimensional NMR spectra for each of said drug cores to be tested for binding to said

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target, wherein said each of said spectra is obtained in
the absence of said target

   ii) mixing together between 2 and 20 of said
drug cores which will not interact with one another;

5   iii) obtaining a one-dimensional NMR spectrum
of said mixture of said drug cores;

   iv) mixing said drug cores with the target,
wherein each of said drug cores is present at a molar
ratio to said target of between 1:1 and 100:1;

10   v) subjecting said mixture of drug cores and
said target to nuclear magnetic resonance for a period of
time sufficient to obtain a one-dimensional spectrum; and

   vi) comparing the spectra obtained in steps
iii) and v) to determine which, if any, of said drug

15 cores has bound to said target.

   Although a ratio of target:drug core of 1:100
is envisioned in the line broadening method of detecting
binding, it is preferred that the ratio be between 1:10
and 1:1.

20   According to another preferred embodiment, the
determination of binding is achieved by the NMR method of
tNOE and comprises the steps of:

   i) mixing the target with the drug core at a
molar ratio of between 1:1 and 1:100.

25   ii) subjecting said mixture to nuclear
magnetic resonance for a period of time sufficient to
obtain a two-dimensional spectrum; and

   iv) analyzing the spectra obtained in step ii)
to determine if said drug core has bound to said target.

30   This method may also be used in conjunction
with a relaxation filter.

   If binding between the drug core and target has
occurred, it will induce a change in the NOE of the cross

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peaks corresponding to the drug core. This will make the sign of the cross peaks the same as the sign of the diagonal peaks. Unbound cores produce a two-dimensional spectrum wherein the signs of the diagonal peaks are

5   usually opposite those of the cross peaks. In those situations where the sign of the unbound drug core is the same as that of the target (i.e., very strong magnetic field or high molecular weight drug core) the amplitude of those peaks will increase significantly if that drug

10 core binds to the target.

The tNOE method may also be utilized with multiple drug cores in a single sample. To do so requires that a one-dimensional NMR spectrum of each individual drug core be obtained, as well as a

15 one-dimensional spectrum of the mixture of drug cores in the absence of the target. The chemical shifts of peaks in the one-dimensional spectra correspond to the chemical shifts of the diagonal peaks observed in a two-dimensional spectrum. Thus, the reference

20 one-dimensional spectra will allow assignment of individual diagonal peaks to a specific drug core. The cross peaks for each individual drug core are then easily identifiable as they appear at the same frequencies as any two diagonal peaks corresponding to that drug core.

25 Although a ratio of drug core:target of 100:1 is envisioned in the tNOE method of detecting binding, it is preferred that the ratio be between 50:1 and 1:1.

According to yet another embodiment, the determination of binding is achieved using the NMR

30 technique of pulsed field gradients and comprises the steps of:

i)   determining a gradient strength that is effective to substantially reduce or eliminate the

20

one-dimensional NMR spectrum of said drug core in the
absence of said target;

        ii)   mixing the target with the drug core at a
molar ratio of between 1:1 and 1:20.

5   iii) subjecting said mixture to nuclear
magnetic resonance for a period of time sufficient to
obtain one-dimensional spectra using the gradient
strength determined in step i); and

        iv)   analyzing the spectrum obtained in step
10   iii), and, if necessary, comparing said spectrum to a
one-dimensional spectrum of said target in the absence of
said drug core at the gradient determined in step i), to
determine if said drug core has bound to said target.

        Although a target:drug core ratio of up to 1:20
15 is envisioned in all of the PFG NMR methods set forth
herein, it is preferable that the ratio be between 1:5
and 1:1. Most preferably, the ratio is about 1:1.

        The free drug core will demonstrate a
substantially reduced spectrum (i.e., peaks with very
20 little amplitude) or no spectrum at all at the gradient
strength utilized. If, however, the drug core has bound
to the protein, the effect of the gradient on reducing or
eliminating the drug core's spectrum is diminished, and
the drug core exhibits a characteristic spectrum. This
25 spectrum is, of course, added to the spectrum of the
target to produce the overall spectrum for the mixture.
In some instances, depending upon the nature of the drug
core and/or the target, one of skill in the art can
ascertain by eye peaks corresponding to the drug core in
30 the spectrum of the mixture. This is because the drug
cores utilized in this invention have characteristically
sharp, narrow peaks, while targets tend to have broader,
more diffuse peaks. In such instances there is no need

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to obtain a spectrum of the target alone at the
determined gradient.

In other instances, peaks corresponding to
bound drug cores may be obscured by the target spectrum.
5   Therefore, the target spectrum in the absence of drug
core needs to be obtained and then subtracted from the
mixture spectrum to reveal peaks corresponding to bound
drug cores. This may be achieved using standard software
utilized in conjunction with NMR techniques.

10   In a preferred embodiment, the PFG NMR
technique is used to quantitate the binding of a drug
core to a target. This method comprises the steps of:

i)   obtaining one-dimensional NMR spectra of
said drug core in the absence of said target at various
15   gradient strengths;

ii)   mixing the target with the drug core at a
molar ratio of between 1:1 and 1:20.

iii) subjecting said mixture to nuclear
magnetic resonance for a period of time sufficient to
20   obtain one-dimensional spectra at the same gradient
strengths utilized in step i; and

iv)   utilizing the spectral data generated in
steps i) and iii) to calculate the K_d between said drug
core and said target.

25   Unlike the line broadening and tNOE methods
described above, the PFG method advantageously quantifies
the binding of the drug core to the target with more
accuracy and substantially less effort. This calculation
is achieved by recognizing that rapid exchange of the
30   drug core between the free and bound states leads to an
apparent diffusion coefficient D_{app}:

$$D_{app} = (1-p_b)D_{free} + p_b(D_{bound}) \qquad [1];$$

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wherein p_b is the fraction of ligands that are bound to
the target; D_{app} is the apparent diffusion coefficient of
the drug core in the presence of the target; D_{free} is
diffusion coefficient of drug core by itself, and D_{bound} is

5   the diffusion coefficient of the bound drug core. The
D_{bound} value is the same as the diffusion coefficient of
the target, and is readily measured. It should be noted
that for higher molecular weight targets, the D_{bound}
contribution to equation [1] becomes increasingly

10 negligible. At sufficiently high molecular weight, D_{app}
is well-approximated by the expression $(1-p_b)D_{free}$, thus
rendering measurement of D_{bound} unnecessary.

        Using equation [1], one solves for the bound
fraction, p_b. Then, from p_b, one can calculate K_d using

15 the formula:

$K_d = (L_{tot}/p_b) \times \{p_b^2 - p_b(1+P_{tot}/L_{tot}) + (P_{tot}/L_{tot})\}$ [2];

where L_{tot} is the total drug core concentration, and P_{tot}
is the total target concentration.

        The value of D_{free} is obtained in step i) by

20 performing a fit of the peak height versus gradient
strength to an exponential decay function, whose exponent
is proportional to the square of the gradient strength.
Thus, the choice of gradients at which to generate
spectra of the drug core alone must be made so that the

25 peak heights corresponding to the drug core decrease in
amplitude as the gradient strength increases. In order
to achieve an accurate measurement of D_{free}, it is
preferred that between 8 and 16 different gradient
strengths be used to generate spectra for the drug core

30 alone.

        The values of D_{app} and D_{bound} are obtained in step
iii) by performing a fit of the peak height versus
gradient strength to the aforementioned exponential decay

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function of the ligand (drug core) peaks and resolvable target peaks (usually attributed to aromatic or methyl groups in the target), respectively.

The use of the PFG NMR method to quantify K_d

5   values of any ligand and a target that fall in the micromolar to millimolar range is yet another aspect of the present invention. According to this embodiment, the invention provides a method of quantifying the dissociation constant between a ligand and a target

10   comprising the steps of:

a)   obtaining a one-dimensional NMR spectrum of said ligand in the absence of said target at various gradient strengths;

b)   mixing the target with the ligand at a

15   molar ratio of between 1:1 and 1:5.

c)   subjecting said mixture to nuclear magnetic resonance for a period of time sufficient to obtain one-dimensional spectra at the same gradient strengths utilized in step i; and

20   d)   utilizing the spectral data generated in steps i) and iii) to calculate the K_d between said ligand and said target.

The term "ligand", as used herein, refers to any molecular entity that is capable of binding to a

25   target.   It is preferred that the ligand have a molecular weight of below about 5 kDa.   It is even more preferred that the ligand have a molecular weight of below about 2 kDa.

According to another embodiment, the invention

30   provides a drug core library -- a plurality of individually compartmentalized compounds and tautomers thereof consisting of:

24

a)   at least one compound of the formula:

;

b)   at least one compound of the formula:
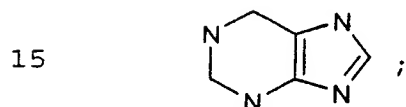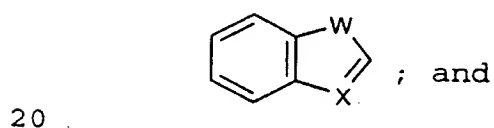

;

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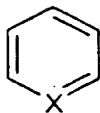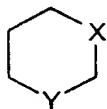
c)   at least one compound of the formula:

;

d)   at least one compound of the formula:
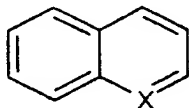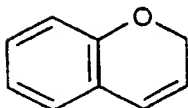
10

;

e)   at least one compound of the formula:

;

15

f)   at least one compound of the formula:

;

g)   at least one compound of the formula:

20

;

h)   at least one compound of the formula:

;

25

25

i)   at least one compound of the formula:
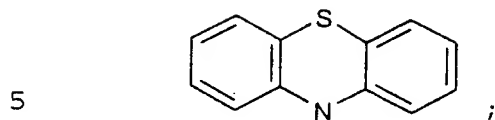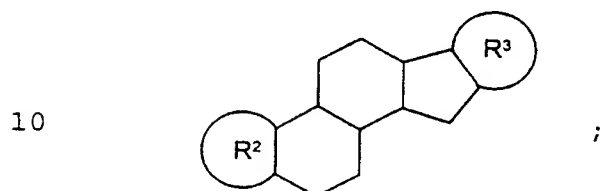
;

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j)   at least one compound of the formula:

;

10

k)   at least one compound of the formula:

;

15

l)   at least one compound of the formula:

; and

20

m)   at least one compound of the formula:

; and optionally includes any of:

,

, or

;

25
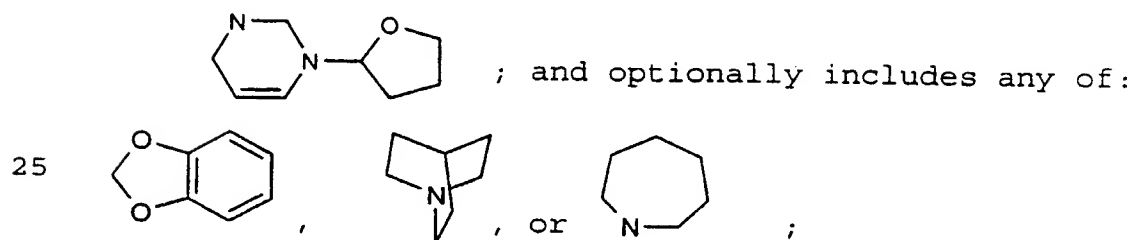
wherein:

V is N or O;

W is N or S;

X is C or N;

30

Y is C, N or O;

Z is selected from a bond, $-CH_2-$, $-NH-$, $-O-$, $-NH-CH_2-$ or $-CH_2-NH-CH_2$

$\mathcal{26}$

R^2 is a 6-membered carbocyclic ring containing 1, 2 or 3 double bonds

R^3, if present, is methylenedioxy; and wherein

any of said compounds is optionally substituted on one or more carbon atoms with one or more substituents independently selected from =O, =S, =N-O-CH_3, -OH, halo, -CN, -(C_1-C_4)-straight or branched alkyl, C_2-alkynyl, -N(R^4)$_2$, -C(O)-R^5, -CH_2C(O)-R^5, -CH(CH_3)C(O)-R^5, -OR^6, -CH_2OH, CH_2NH_2, -CF_3, -S(O)$_2NH_2$, -S(O)$_2$OH or -OCH_2CHOHCH_2NH(C_1-C_4)-straight or branched alkyl;

any of said compounds is optionally substituted on one or more nitrogen atoms, if present, with a -(C_1-C_3)-straight or branched alkyl or -(CH_2)$_{1-3}$-OH; and

any of said compounds is optionally substituted on a sulfur atom, if present, with =O; wherein:

each R^4 is independently selected from H, O, C(O)-CH_3 or -(C_1-C_3)-straight or branched alkyl;

each R^5 is selected from (CH_2)$_{0-3}$-OH, O-(C_1-C_3)-straight or branched alkyl, NH_2, or (C_1-C_3)-straight or branched alkyl; and

each R^6 is selected from -(C_1-C_3)-straight or branched alkyl, or C(O)-(C_1-C_3)-straight or branched alkyl.

More preferably, the plurality of individually compartmentalized compounds consists of:

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and tautomers thereof; wherein each of said compounds is optionally substituted as set forth above.
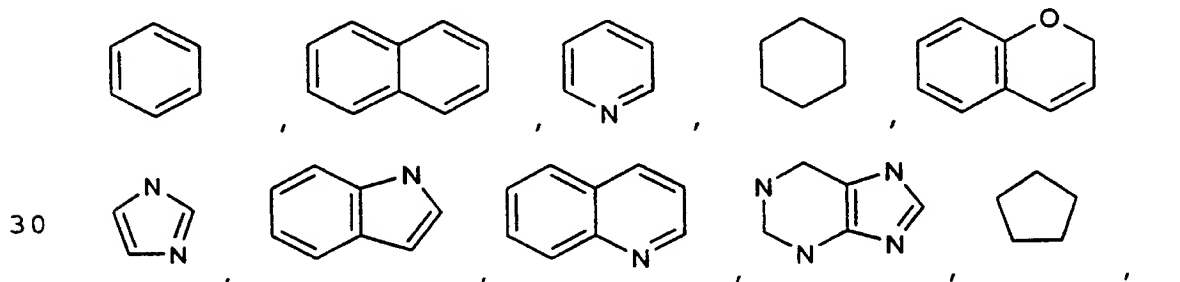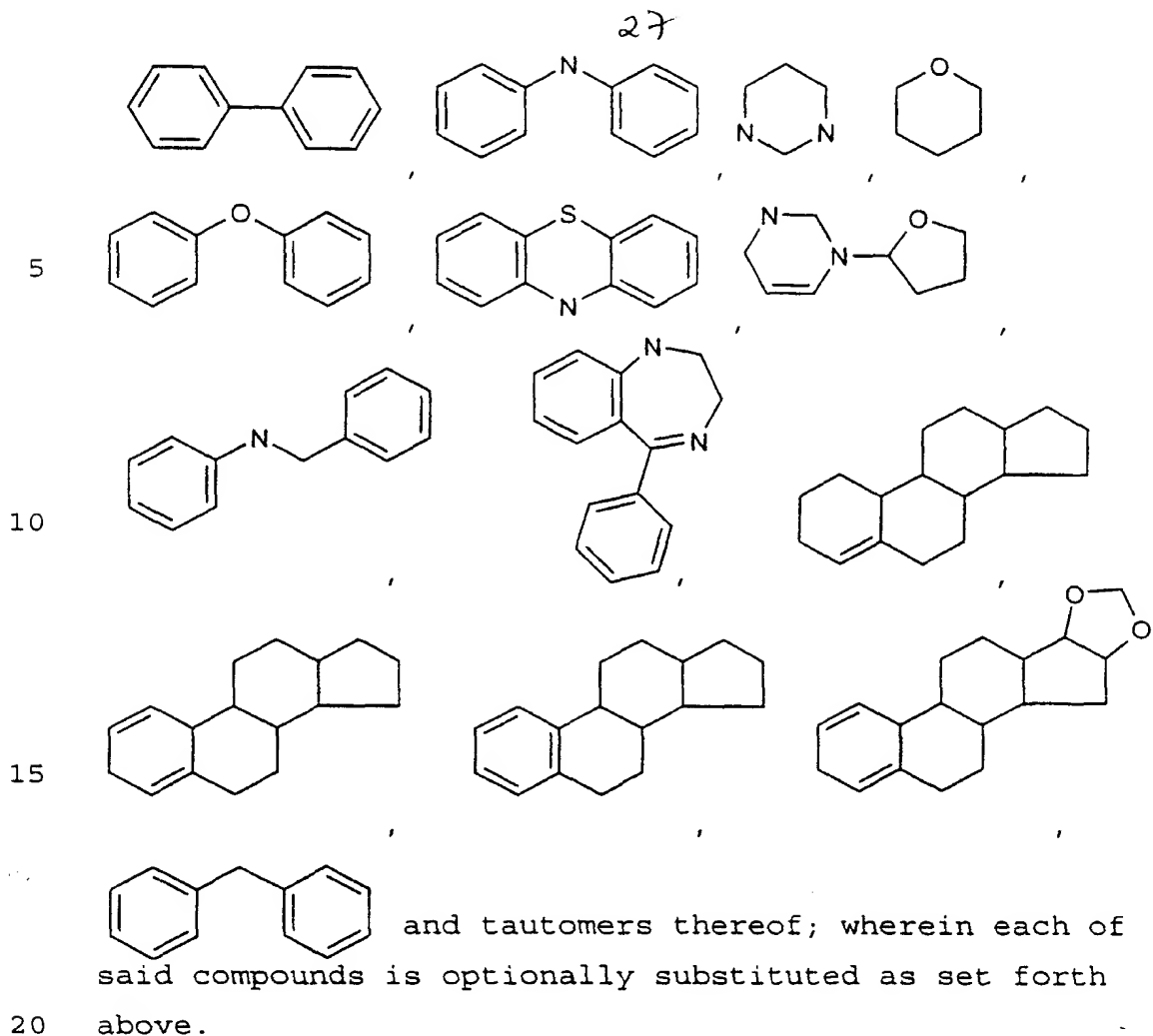
According to another preferred embodiment, the optional substituents on one or more carbon atoms are independently selected from $=O$, $-CH_3$, $-OH$, $-OCH_3$, $-Cl$, $-NH_2$, $-C(O)OH$, $-F$, $-CH_2OH$, $-CH_2CH_3$, $-OC(O)CH_3$, $-NO_2$, $-N(CH_3)_2$, $-CF_3$, $-C(O)NH_2$, $-C(O)OCH_3$, $-C(O)OCH_2CH_3$, $-CH(CH_3)_2$, $-S(O)_2NH_2$, $-C(O)CH_3$, $-CN$, $-Br$, $-I$, $-S(O)_2OH$, $-OCH_2CH_3$, $-CH_2C(O)OH$, $-OC(O)CH_2CH_3$, $-CH_2CH(CH_3)_2$, $-C(O)CH_2OH$, $-N(H)C(O)CH_3$, $-C(CH_3)_3$, $=S$, $-CH_2NH_2$, $-OCH_2CH(OH)CH_2N(H)C(CH_3)_3$, $-N(H)CH_3$, $-CH(CH_3)C(O)OH$, $-C\equiv CH$, $-(CH_2)_2CH_3$, $-CH_2C(O)NH_2$, $-OCH_2CH(OH)CH_2N(H)CH(CH_3)_2$, or $=N-OCH_3$;

28

the optional substituents on one or more nitrogen atoms, if present, are independently selected from $-CH_3$, $-(CH_2)_2OH$ or $-CH_2CH_3$; and

the optional substituent on a sulfur atom, if
5   present, is =O.

Even more preferred is that the substituents attached to a carbon atom are independently selected from =O, $-OCH_3$, $-OH$, $-NH_2$, $-C(O)OH$, $-S(O)_2OH$, $-S(O)_2NH_2$, $-CH_2OH$ or $-C(O)NH_2$; and the substituent attached to a nitrogen
10   atom is CH_3.

Preferably, the drug core contains from 0 to 3 total substituents.

The term "individually compartmentalized", as used herein, refers to each compound being physically
15   separate and apart from one another. The term is intended to encompass each compound being present in a separate container (test tube, vial, or other storage medium commonly used in the art); each compound being attached to a separate bead or other inert, solid media;
20   each compound being present in a separate well in a multi-well plate; and any other means for storing the compounds physically separate from one another.

As discussed in detail, above, the plurality of individually compartmentalized compounds represents
25   moieties that are present in known drugs. Thus, this plurality of compounds represents a small, distinct library of chemical entities each possessing desirable properties for engineering into a drug. These include solubility, bioavailability, ease and low cost of
30   synthesis (including low cost of starting materials and the ability to produce the final product using few synthetic steps), low toxicity, and chemical and metabolic stability.

29

Because the library of this invention consists of cores and side chains that are present in commercially available drugs, any weak binders detected therein will necessarily have desirable druglike qualities. Moreover, the small size of the library makes screening less time and labor intensive. Finally, the diverse nature of the cores and side chains in the library means that multiple "hits" (i.e., weak binders) allows the flexibility and advantages of pursuing several compound classes at once.

Once a drug core is identified as binding to a target, it can then be modified by any or all of the following: linking with other binding cores, addition of appendages, fusion with other ring structures, addition of substituents and addition of other chemical groups to optimize interaction with the target and produce a drug that is safe and effective to administer to a mammal.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

EXAMPLE 1
Identification of Drug Cores that Bind to p38 MAP Kinase

To demonstrate the feasibility of using DLB and tNOE techniques on a typical drug target, a number of different drug cores were screened against the medium sized protein p38 MAP kinase.

All the spectra were acquired at 5° C using a Bruker DMX 500 MHz NMR spectrometer. NOESY spectra were collected using 16 transients with 2048 points in ω_2 and 400 points in ω_1 and a mixing time of 400 ms for the drug

30

core mixture in the absence of target and 100 ms for the
drug core mixture in the presence of p38. A spin echo
with an echo delay of 5 ms was used as a relaxation
filter before the t_1 evolution period in all experiments.

5   Samples contained 1 mM of each drug core, 0.2 mM p38 MAP
kinase, 25 mM deutero-Tris, 10% (v/v) deutero-glycerol,
20 mM deutero-dithiothreitol at $pD^* = 8.4$.

Figure 1 illustrates line broadening,
suppression of fine structure and attenuation of ligand

10 resonance peak height due to the relaxation filter in the
presence of the protein, indicating that 2-phenoxy
benzoic acid binds to the protein. Close comparison of
the two spectra indicate that 2-phenoxy benzoic acid
binds to p38 MAP kinase while nicotinic acid does not.

15   Figure 2 demonstrates that nicotinic acid ('X')
and 2-phenoxy benzoic acid ('Y') in the mixture without
p38 have weak NOE cross peaks, with sign opposite to that
of the diagonal peaks (panel A) (this is further
illustrated by slices through the 2D spectra shown in

20 Figure 3). Panel B shows that in the presence of p38,
the cross peaks remain opposite in sign from the diagonal
peaks for nicotinic acid, indicating this compound does
not bind. However, the sign of the cross peaks of 2-
phenoxy benzoic are now the same as the diagonal peaks,

25 indicating this compound binds to the protein.

                        EXAMPLE 2

      K_D Determination of 2-phenoxybenzoic acid to p38

Underline rendering: K$_D$ Determination of 2-phenoxybenzoic acid to p38

Two NMR samples were prepared for this study:
one sample containing only the 2-phenoxybenzoic acid at

30 0.5 mM, and another having both p38 and 2-phenoxybenzoic
acid at 0.1 mM and 0.2 mM, respectively. The buffer for

_31_

both samples consisted of 20 mM deutero-DTT, 25 mM
deutero-Tris, 10% (v/v) deutero-glycerol. The pD* was
set to 8.4. Diffusion coefficients for both the protein
and 2-phenoxybenzoic acid were obtained from the p38/2-

5   phenoxybenzoic acid sample, due to the fortuitous
appearance of resolved aromatic resonances of p38.

The one-dimensional PFG NMR experiment used for
the diffusion measurements was the water-sLED experiment
shown in Fig. 4 and described in A. S. Altieri et al., _J._

10   _Am. Chem. Soc._, 117, pp. 7566-7567 (1995), the disclosure
of which is herein incorporated by reference. All
experiments were carried out at 295K on a Bruker DMX-
500MHz spectrometer. All gradient pulses were
rectangular shaped and applied along the z-axis. The

15   critical gradients were the phase-encoding/decoding
gradients, corresponding to the two shaded gradient
pulses immediately prior and following the "T" period.
For both samples, 23 data sets were recorded
corresponding to increasing strengths of the phase

20   encoding/decoding gradients. These strengths ranged from
1.3 gauss/cm to 29.4 gauss/cm, in steps of 1.3 gauss/cm.

Molecular diffusion between the phase-encoding
and phase-decoding gradients attenuated the peak heights
of the resulting spectra. This attenuation exacerbates

25   with increasing gradient strength. This effect is
illustrated in Figure 5. Severe peak attenuation occurs
for the ligand signals (four larger resonances on the
right-hand side), while little attenuation occurs for the
p38 aromatic resonances (smaller sharp resonances on the

30   left-hand side).

Denoting a given peak integral by I, the
attenuation is described by the decay equation:

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$$I = A \exp\{-DK^2\}. \qquad [3]$$

In the above expression, D is the desired diffusion coefficient, A is the peak integral in the absence of the two phase encoding/decoding gradients, and

5   K^2 is the a factor proportional to the square of the gradient strength given by $\gamma^2 \delta^2 G_z^2 (\Delta - \delta/3)$. Fitting a data file consisting of peak integrals versus K^2 to equation 3 gives the diffusion coefficient, D, and the prefactor, A. Examples of these fits are shown in Figure 6.

10   Data sets for both samples were Fourier-transformed using commercial software (Xwinnmr 1.2, Bruker Instruments, Billerica, MA). The resulting peaks were integrated and then listed with the corresponding values for the phase-encoding/decoding gradients. These

15   gradient values were converted into K^2 values, as defined above in equation 3. Diffusion coefficients were determined by using the well-known Levenburg-Marquardt algorithm to fit the resulting data files to equation 3. The fits are shown in Figure 6. The 2-phenoxybenzoic

20   acid sample yielded D_{free} = 0.438 cm²/sec. The resolved ligand resonances of p38/2-phenoxybenzoic acid sample yielded D_{app} = 0.308 cm²/sec, while the resolved p38 aromatic resonances of the same sample yielded D_{bound} = 0.044 cm²/sec.

25   Inserting these values into equation 1, set forth above, a bound fraction of p_b = 0.33. Insertion of this p_b value into equation 2, set forth above, gave a K_d of 70 μM.

While we have hereinbefore presented a number

30   of embodiments of this invention, it is apparent that our basic construction can be altered to provide other embodiments of this invention. Therefore, it will be

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appreciated that the scope of this invention is to be
defined by the claims appended hereto rather than the
specific embodiments which have been presented
hereinbefore by way of example.

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CLAIMS

We claim:

1.   A method of identifying a drug core suitable for a given target comprising the steps of:

    a.   providing a drug core consisting of a

5 cyclic structure or a tautomer thereof selected from:
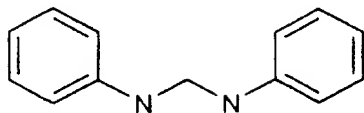
35

; wherein said cyclic structure is
optionally substituted at:

      i)   one or more carbon atoms with one or more
substituents independently selected from $=O$, $-CH_3$,
$-OH$, $-OCH_3$, $-Cl$, $-NH_2$, $-C(O)OH$, $-F$, $-CH_2OH$,
$-CH_2CH_3$, $-OC(O)CH_3$, $-NO_2$, $-N(CH_3)_2$, $-CF_3$, $-C(O)NH_2$,
$-C(O)OCH_3$, $-C(O)OCH_2CH_3$, $-CH(CH_3)_2$, $-S(O)_2NH_2$,
$-C(O)CH_3$, $-CN$, $-Br$, $-I$, $-S(O)_2OH$, $-OCH_2CH_3$,
$-CH_2C(O)OH$, $-OC(O)CH_2CH_3$, $-CH_2CH(CH_3)_2$, $-C(O)CH_2OH$,
$-NH-C(O)CH_3$, $-C(CH_3)_3$, $=S$, $-CH_2NH_2$,
$-OCH_2CHOHCH_2NHC(CH_3)_3$, $-NHCH_3$, $-C(CH_3)C(O)OH$, $-C{\equiv}CH$,
$-(CH_2)_2CH_3$, $-CH_2C(O)NH_2$, $-OCH_2CHOHCH_2NHCH(CH_3)_2$,
$=N-OCH_3$, or $-OCH_2CH_3$

      ii) one or more nitrogen atoms, if present,
with a substituent independently selected from $-CH_3$,
$-(CH_2)_2OH$ or $-CH_2CH_3$; and

      iii) a sulfur atom, if present, with $=O$; and

   c)   determining whether any one of said drug cores
binds to said target.

     2.   The method according to claim 1, wherein:
said cyclic structure is selected from:

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, or tautomers thereof; and

said optional substituents on one or more carbon atoms are independently selected from =O, -CH$_3$, -OH, -OCH$_3$, -Cl, -NH$_2$, -C(O)OH, -F, -CH$_2$OH, -CH$_2$CH$_3$, -OC(O)CH$_3$, -NO$_2$, -N(CH$_3$)$_2$, -CF$_3$, -C(O)NH$_2$, -C(O)OCH$_3$, -C(O)OCH$_2$CH$_3$, -CH(CH$_3$)$_2$, -S(O)$_2$NH$_2$, -C(O)CH$_3$, -CN, -Br, -I, -S(O)$_2$OH, -OCH$_2$CH$_3$, -CH$_2$C(O)OH, -OC(O)CH$_2$CH$_3$, -CH$_2$CH(CH$_3$)$_2$, -C(O)CH$_2$OH, -NH-C(O)CH$_3$, -C(CH$_3$)$_3$, =S, -CH$_2$NH$_2$, -OCH$_2$CHOHCH$_2$NHC(CH$_3$)$_3$, -NHCH$_3$, -C(CH$_3$)C(O)OH, -C≡CH, -(CH$_2$)$_2$CH$_3$, -CH$_2$C(O)NH$_2$, -OCH$_2$CHOHCH$_2$NHCH(CH$_3$)$_2$, or =N-OCH$_3$.

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3.   The method according to claim 2, wherein:
said optional substituents on one or more carbon
atoms are independently selected from =O, -OCH$_3$, -OH,
-NH$_2$, -C(O)OH, -S(O)$_2$OH, -S(O)$_2$NH$_2$, -CH$_2$OH or -C(O)NH$_2$; and
5   said optional substituent attached to a nitrogen
atom is CH$_3$.

4.   The method according to any one of claims
1 to 3, wherein determining whether the drug core binds
10   to said target comprises the steps of:
        i)   obtaining a one-dimensional NMR spectrum
of said drug core in the absence of said target;
        ii)   mixing the target with the drug core at a
molar ratio of between 1:1 and 1:100;
15   iii) subjecting said mixture to nuclear
magnetic resonance for a period of time sufficient to
obtain a one-dimensional spectrum; and
        iv)   comparing the spectra obtained in steps i)
and iii) to determine if said drug core has bound to said
20   target.

5.   The method according to any one of claims
1 to 3, wherein more than one of said drug cores is
tested simultaneously for binding to said target; and
25   wherein determining whether the drug core binds to said
target comprises the steps of:
        i)   obtaining one-dimensional NMR spectra for
each of said drug cores to be tested for binding to said
target, wherein said spectra is obtained in the absence
30   of said target
        ii)   mixing together between 2 and 20 of said
drug cores which will not react with one another;

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iii) obtaining a one-dimensional NMR spectrum of said mixture of said drug cores;

iv) mixing said drug cores with the target, wherein each of said drug cores is present at a molar

5 ratio to said target of between 1:1 and 100:1;

v) subjecting said mixture of drug cores and said target to nuclear magnetic resonance for a period of time sufficient to obtain a one-dimensional spectrum; and

vi) comparing the spectra obtained in steps

10 iii) and v) to determine which, if any, of said drug cores has bound to said target.

6. The method according to any one of claims 1 to 3, wherein determining whether the drug core binds

15 to said target comprises the steps of:

i) mixing the target with the drug core at a molar ratio of between 1:1 and 1:100.

ii) subjecting said mixture to nuclear magnetic resonance for a period of time sufficient to

20 obtain a two-dimensional spectrum; and

iii) analyzing the spectrum obtained in step ii) to determine if said drug core has bound to said target.

25 7. The method according to any one of claims 1 to 3, wherein more than one of said drug cores is tested simultaneously for binding to said target; and wherein determining whether the drug core comprises the steps of:

30 i) obtaining one-dimensional NMR spectra for each of said drug cores to be tested for binding to said

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target, wherein said spectra is obtained in the absence
of said target

        ii)   mixing together between 2 and 20 of said
drug cores which will not react with one another;

5   iii) obtaining a one-dimensional NMR spectrum
of said mixture of said drug cores;

        iv)   mixing said drug cores with the target,
wherein each of said drug cores is present at a molar
ratio to said target of between 1:1 and 100:1;

10   v)   subjecting said mixture of drug cores and
said target to nuclear magnetic resonance for a period of
time sufficient to obtain a two-dimensional spectrum; and

        vi)   comparing the spectra obtained in steps
iii) and v) to determine which, if any, of said drug

15   cores has bound to said target.

        8.   The method according to any one of claims
1 to 3, wherein determining whether the drug core binds
to said target comprises the steps of:

20   i)   determining a gradient strength that is
effective to substantially reduce or eliminate the
one-dimensional NMR spectrum of said drug core in the
absence of said target;

        ii)   mixing the target with the drug core at a

25   molar ratio of between 1:1 and 1:20.

        iii) subjecting said mixture to nuclear
magnetic resonance for a period of time sufficient to
obtain one-dimensional spectra using the gradient
strength determined in step i); and

30   iv)   analyzing the spectrum obtained in step
iii), and, if necessary, comparing said spectrum to a
one-dimensional spectrum of said target in the absence of

40

said drug core at the gradient determined in step i), to determine if said drug core has bound to said target.

9.   The method according to any one of claims 1 to 3, wherein the determination of whether the drug core binds to said target is quantitative, and comprises the steps of:

i)   obtaining one-dimensional NMR spectra of said drug core in the absence of said target at various gradient strengths;

ii)   mixing the target with the drug core at a molar ratio of between 1:1 and 1:20.

iii) subjecting said mixture to nuclear magnetic resonance for a period of time sufficient to obtain one-dimensional spectra at the same gradient strengths utilized in step i; and

iv)   utilizing the spectral data generated in steps i) and iii) to calculate the K_d between said drug core and said target.

10.   A method of calculating the dissociation constant, K_d, between a ligand and a target comprising the steps of:

a)   obtaining a one-dimensional NMR spectra of said ligand in the absence of said target at various gradient strengths;

b)   mixing the target with the ligand at a molar ratio of between 1:1 and 1:20.

c)   subjecting said mixture to nuclear magnetic resonance for a period of time sufficient to obtain one-dimensional spectra at the same gradient strengths utilized in step i; and

41

    d)   utilizing the spectral data generated in steps i) and iii) to calculate the K_d between said ligand and said target.

5   11.   A plurality of individually compartmentalized compounds consisting of:

    a)   at least one compound of the formula:

;
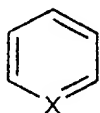
    b)   at least one compound of the formula:

10

;
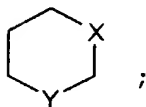
    c)   at least one compound of the formula:
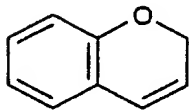
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;
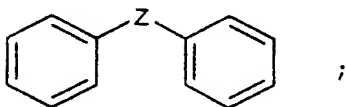
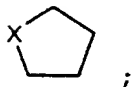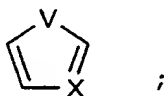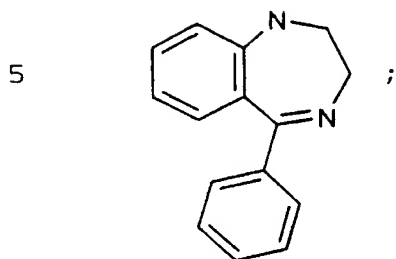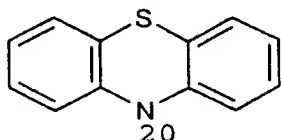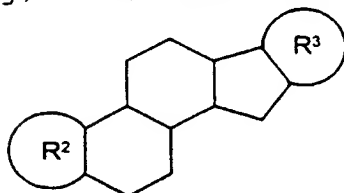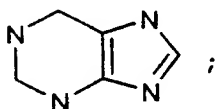   d)   at least one compound of the formula:

;

20
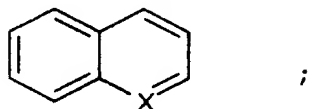
    e)   at least one compound of the formula:

;

25   f)   at least one compound of the formula:

;

    g)   at least one compound of the formula:

30

;

42

h)   at least one compound of the formula:

5

;

i)   at least one compound of the formula:

15

;

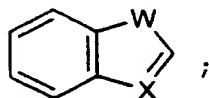j)   at least one compound of the formula:

25

;

k)   at least one compound of the formula:

30

;
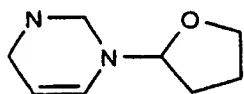
l)   at least one compound of the formula:

;

n)   at least one compound of the formula:

35

; and tautomers thereof; and
optionally includes any of:

40

, or

;

wherein:

V is N or O;

43

W is N or S;

X is C or N;

Y is C, N or O;

Z is selected from a bond, $-CH_2-$, $-NH-$, $-O-$ or

5 $-N-CH_2-$

R^2 is a 6-membered carbocyclic ring containing 1, 2

or 3 double bonds

R^3, if present, is methylenedioxy; and wherein

any of said compounds is optionally substituted on

10 one or more carbon atoms with one or more substituents

independently selected from $=O$, $-OH$, halo, $-CN$,

$-(C_1-C_3)-$straight or branched alkyl, $-N(R^4)_2$, $-C(O)-R^5$,

$-OR^6$, $-CH_2OH$, $-CF_3$, $-S(O)_2NH_2$;

any of said compounds is optionally substituted on

15 one or more nitrogen atoms, if present, with a

$-(C_1-C_3)-$straight or branched alkyl; and

any of said compounds is optionally substituted on a

sulfur atom, if present, with $=O$;

wherein each R^4 is independently selected from H, O,

20 or $-(C_1-C_3)-$straight or branched alkyl;

each R^5 is selected from OH, $O-(C_1-C_3)-$straight or

branched alkyl, NH_2, or $(C_1-C_3)-$straight or branched

alkyl; and

each R^6 is selected from $-(C_1-C_3)-$straight or

25 branched alkyl, or $C(O)-(C_1-C_3)-$straight or branched

alkyl.

12. The plurality of individually

compartmentalized compounds according to claim 11,

30 wherein said compounds consist of:
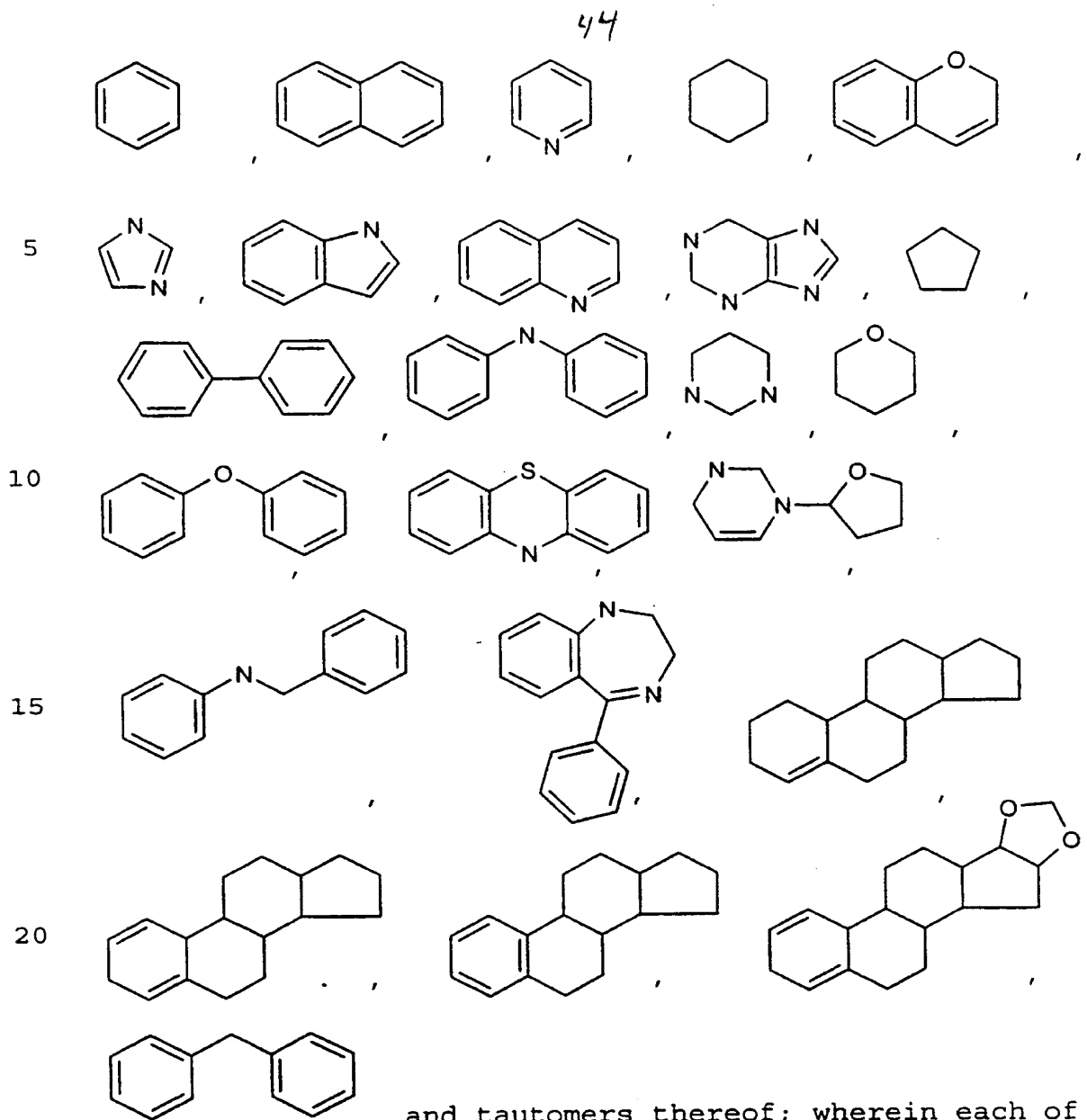
44

, and tautomers thereof; wherein each of
said compounds is optionally substituted as set forth in
claim 11.

13. The plurality of compounds according to
claim 12, wherein:

the optional substituents on one or more carbon
atoms are independently selected from =O, -CH₃, -OH,
-OCH₃, -Cl, -NH₂, -C(O)OH, -F, -CH₂OH, -CH₂CH₃, -OC(O)CH₃,

45

$-NO_2$, $-N(CH_3)_2$, $-CF_3$, $-C(O)NH_2$, $-C(O)OCH_3$, $-C(O)OCH_2CH_3$,

$-CH(CH_3)_2$, $-S(O)_2NH_2$, $-C(O)CH_3$, $-CN$, $-Br$, $-I$, $-S(O)_2OH$, $-$

OCH_2CH_3, $-CH_2C(O)OH$, $-OC(O)CH_2CH_3$, $-CH_2CH(CH_3)_2$, $-C(O)CH_2OH$,

$-N(H)C(O)CH_3$, $-C(CH_3)_3$, $=S$, $-CH_2NH_2$,

5      $-OCH_2CH(OH)CH_2N(H)C(CH_3)_3$, $-N(H)CH_3$, $-CH(CH_3)C(O)OH$, $-C\equiv CH$,

$-(CH_2)_2CH_3$, $-CH_2C(O)NH_2$, $-OCH_2CH(OH)CH_2N(H)CH(CH_3)_2$, or

$=N-OCH_3$;

the optional substituents on one or more nitrogen

atoms, if present, are independently selected from $-CH_3$,

10   $-(CH_2)_2OH$ or $-CH_2CH_3$; and

the optional substituent on a sulfur atom, if

present, is $=O$.

14.   The plurality of compounds according to

15   claim 13, wherein:

the optional substituents attached to a carbon atom

are independently selected from $=O$, $-OCH_3$, $-OH$, $-NH_2$,

$-C(O)OH$, $-S(O)_2OH$, $-S(O)_2NH_2$, $-CH_2OH$ or $-C(O)NH_2$;

the optional substituent attached to a nitrogen atom

20   is CH_3; and

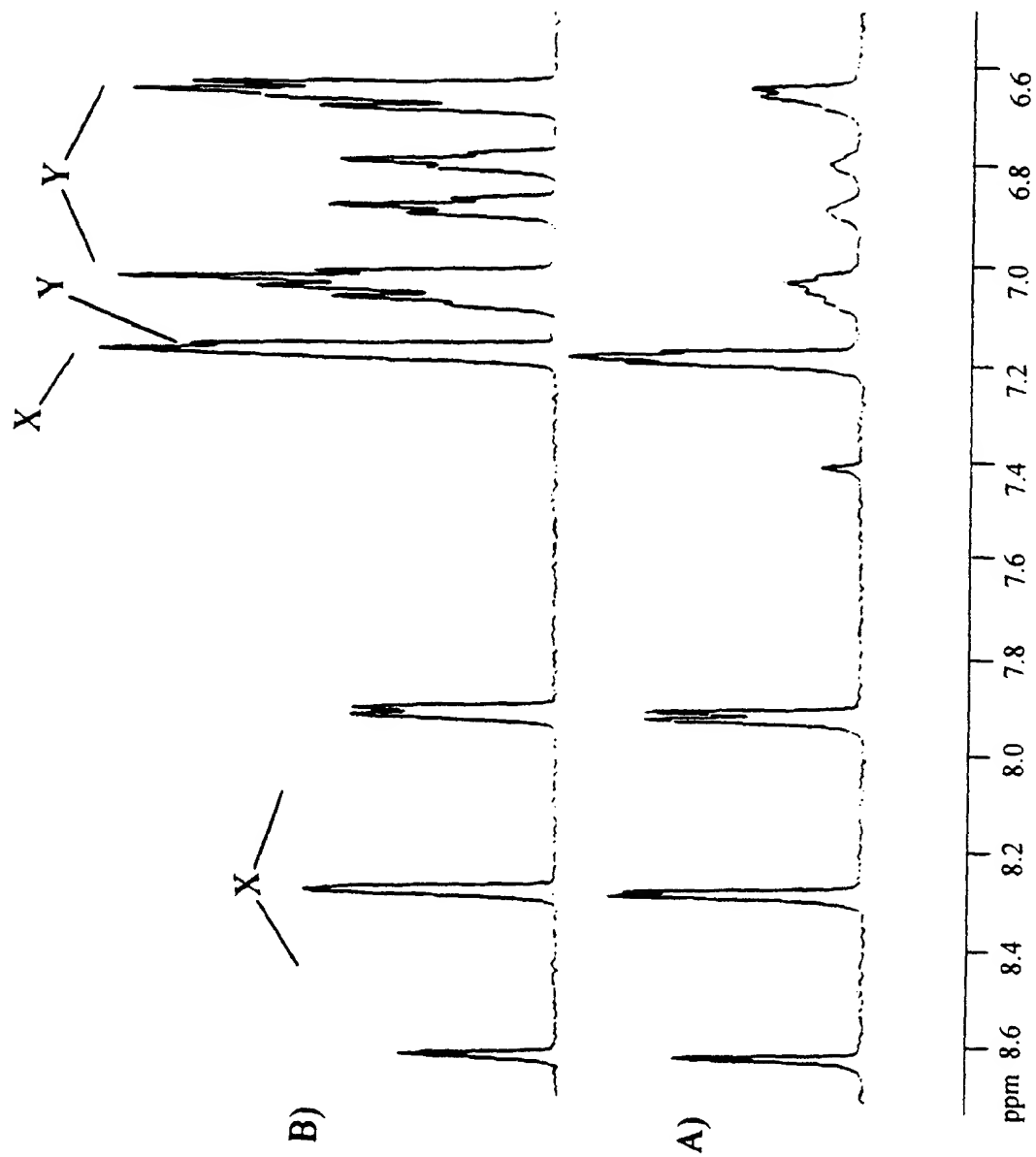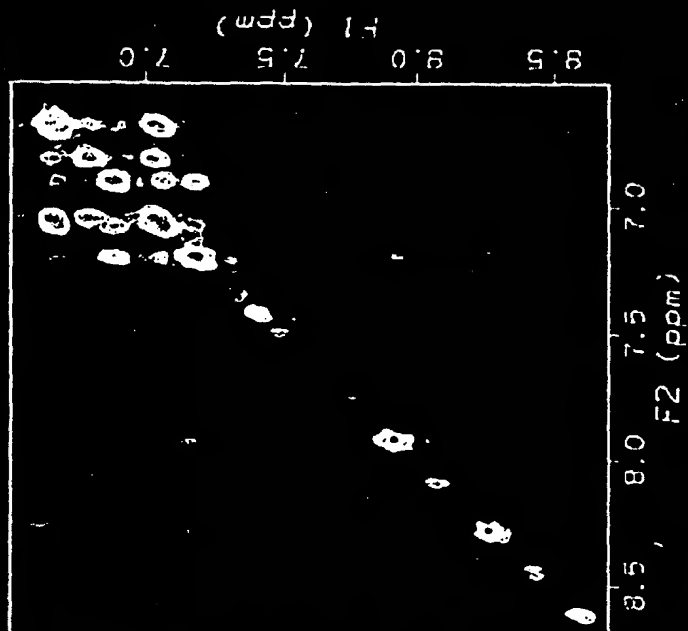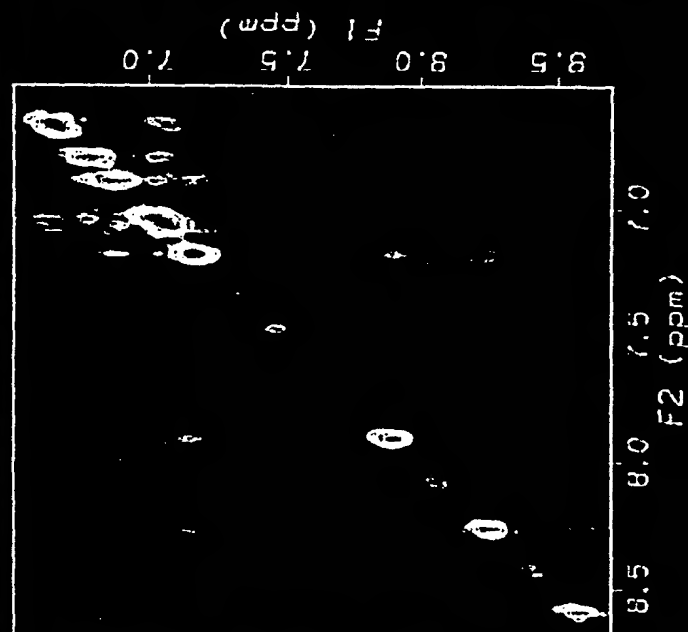the optional substituent attached to a sulfur atom

is $=O$.

FIGURE 1

FIGURE 2

A — free ligands (2)
cross peaks (-)
diagonal peaks (+)

B — ligands (2) + p38 MAP Kinase
Cross peaks invert sign (+) ⇒ binding
Cross peaks remain (-) ⇒ no binding

FIGURE 3

FIGURE 4

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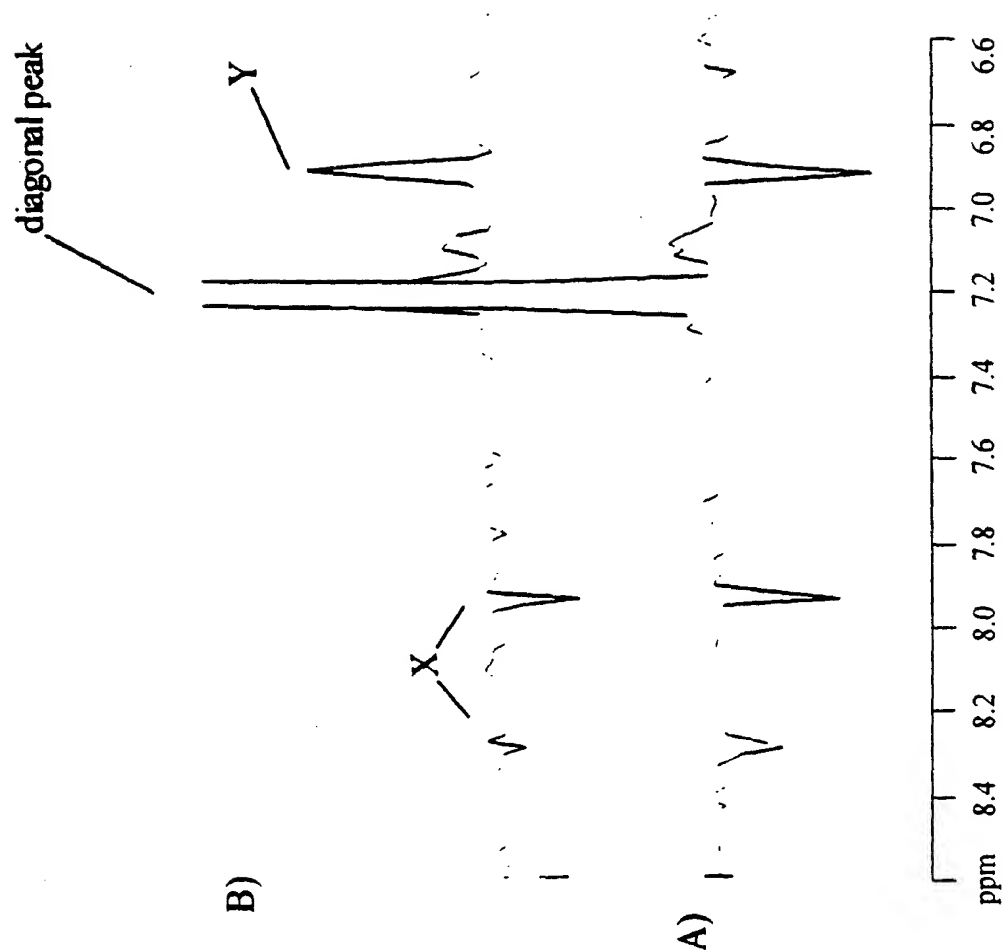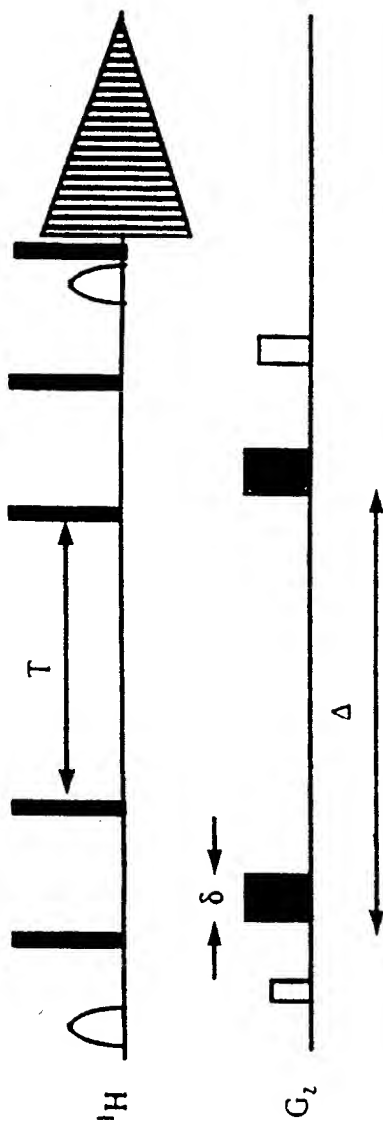
FIGURE 5

FIGURE 6

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   G01N24/08   G01N24/12   G01N33/53

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   G01N   C12Q   C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 18471 A (ABBOTT LAB) 22 May 1997 cited in the application see page 3, line 1 - line 12 ---	6
A	WO 96 22530 A (CHIRON CORP) 25 July 1996 see the whole document ---	4-10
A	US 5 001 427 A (FUJIWARA TOSHIMICHI) 19 March 1991 see the whole document ---	4-10
	-/--	

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 October 1998	01/12/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hoekstra, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication.where appropriate. of the relevant passages	Relevant to claim No.
A	URRY D W ET AL: "Cesium-133 NMR longitudinal relaxation study of ion binding to the Gramicidin transmembrane channel" JOURNAL OF MAGNETIC RESONANCE, 15 OCT. 1985, USA, vol. 65, no. 1, pages 43-61, XP002081761 ISSN 0022-2364 see the whole document ---	4-10
A	BAIANU I C ET AL: "NMR study of chloride ion interactions with thylakoid membranes" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, JUNE 1984, USA, vol. 81, no. 12, pages 3713-3717, XP002081762 ISSN 0027-8424 see the whole document -----	4-10

2

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first she_t)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:   1-3 (part), 11-14
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out, specifically:

   The claimed invention relates, as is derived from the of claims read in the
   light of the description, to identifying lead molecules that are able to
   bind to any biologically important molecule which is capable of binding to
   another molecule, briefly referred to as "targets".

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all
       searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
       of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report
       covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
       restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

determine). In consequence claims 1-3 have only been searched insofar
they encompass the subject-matter of claim 4.

Again without prejudice to the opinion of the IPEA or the decision of the
granting authority the ISA notifies that the description relating to the
subject-matter of claims 11-14 as well as these claims themselves do not
comply with the prescribed requirements of Articles 5 and 6, in particular
Rule 5.1(a)(iii) and Rule 6.3(a) PCT. The description does not disclose the
subject-matter of claims 11-14 as an invention , i.e. in such terms that a
technical problem and a solution thereto can be understood. It is clearly
derivable from the description that the "use", if any, of an individual
compound depends on the "target" used to identify the lead compound,
moreover, the ISA has no indication of the definition of the matter for which
protection is sought in view of the wording of claim 11-14 ("A plurality of
individually compartmentalized compounds...", i.e. a rack with at least wo
test-tubes?, a laboratory comprising at least two test tubes?, etc.) and would
hence be unable to assess novelty and inventiveness on the basis any
document disclosing any individual compound or a combination of these
compounds falling under the Markush definitions of these claims.

INTERNATIONAL SEARCH REPORT

Information on patent family members

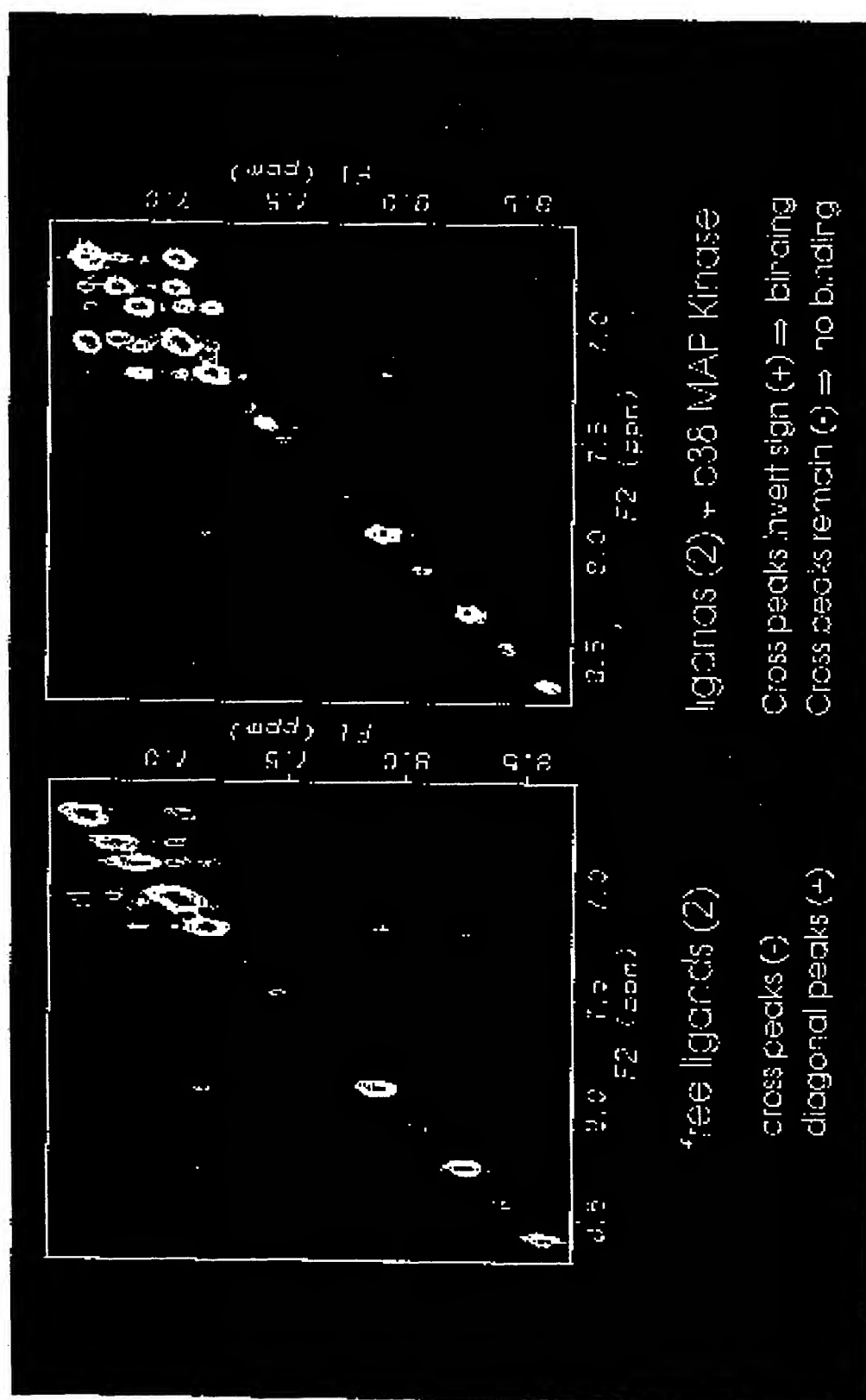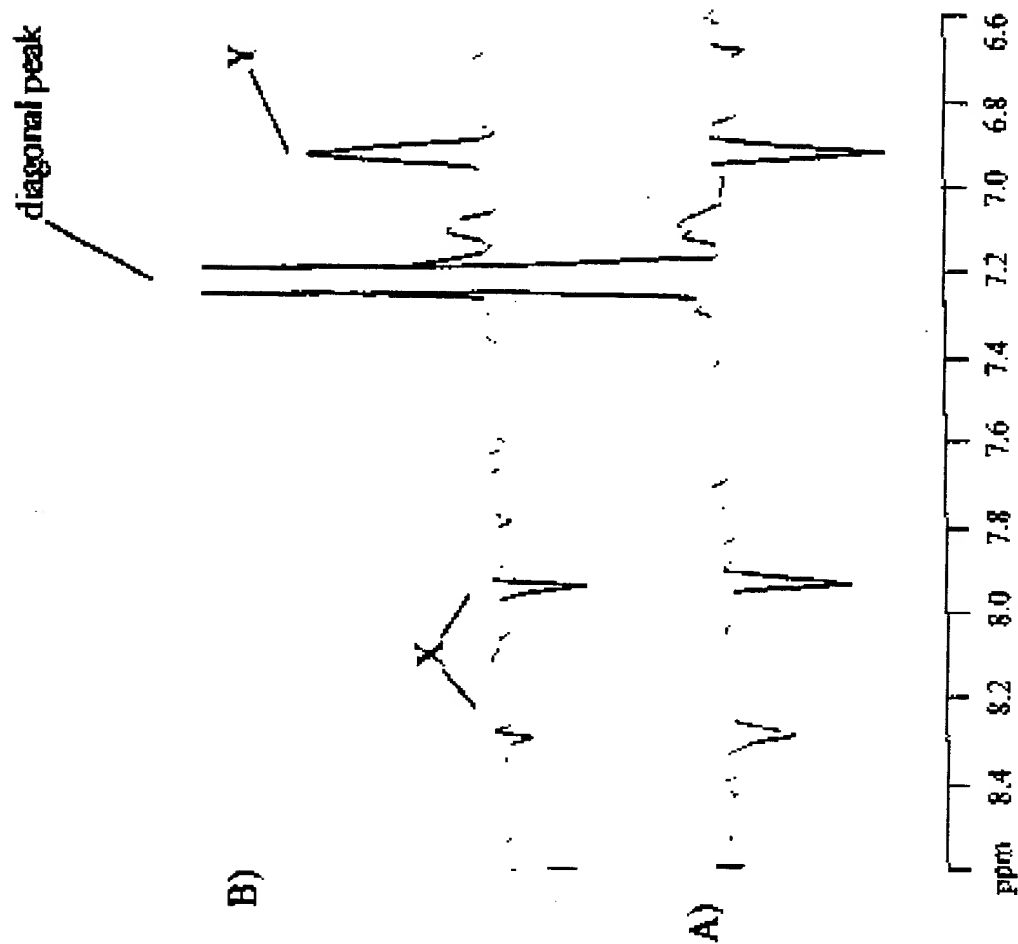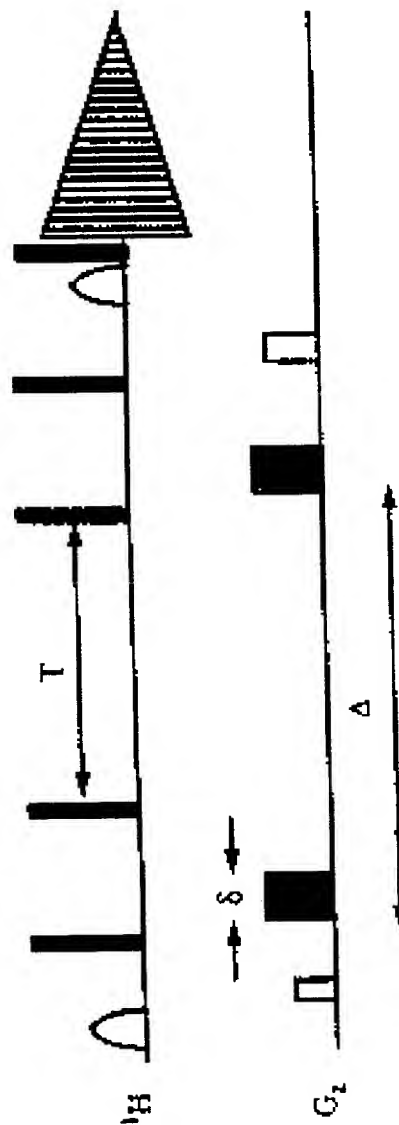| Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9718471	A	22-05-1997	US	5698401 A	16-12-1997
			AU	7732896 A	05-06-1997
			EP	0866967 A	30-09-1998
			US	5804390 A	08-09-1998
WO 9622530	A	25-07-1996	AU	4963796 A	07-08-1996
			CA	2209988 A	25-07-1996
			EP	0804729 A	05-11-1997
US 5001427	A	19-03-1991	JP	2114188 A	26-04-1990

FIGURE 1
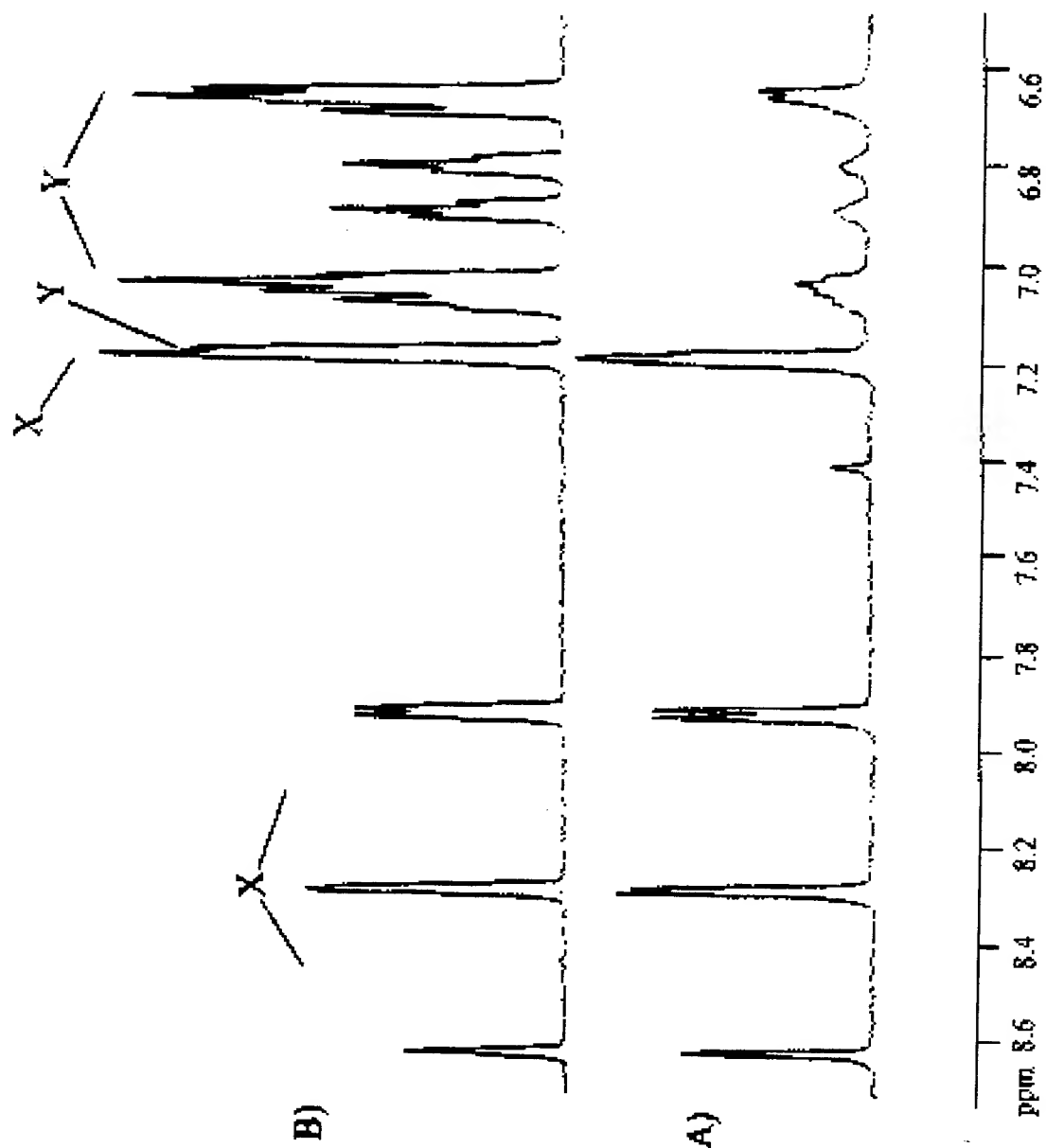
FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6